US ERA ARCHIVE DOCUMENT

### 28-DAY FRESHWATER SEDIMENT TOXICITY TESTS WITH THE AMPHIPOD (HYALELLA AZTECA)

Revised: 7 February 2003

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### I. PURPOSE

This method is designed to measure the lethal and sublethal toxicity of freshwater sediments to the amphipod *Hyalella azteca* using survival and growth as the endpoint following a 28-day exposure.

#### II. EQUIPMENT

- Proven source of clean freshwater water (e.g. moderately hard, synthetic water, EPA 1993)
- Environmental chamber maintained at 23 ± 1°C
- . Cold room maintained at 4°C to hold sediment samples prior to test initiation
- Thermometer, pH meter, dissolved oxygen (D.O.) meter, conductivity meter, dissecting light microscope and analytical balance
- Spectrophotometer and Hach kit for measuring ammonia levels.
- 1-L glass jars with lids (or equivalent) to serve as test exposure chambers (5 jars per test site, reference, and control); holes in lids to permit aeration and feeding
- 0.5-mm and 1.0-mm Nitex mesh screens, transfer pipets, and glass holding bowls
- . Aeration source, 1.0-ml pipets, and airline tubing
- . Test, control, and reference sediments
- . 30-ml plastic cups for holding animals; freshwater squirt bottle
- Safety equipment lab coats, eye protection, gloves, and respirator as required

#### III. TEST PROCEDURE

#### A. Pre-test Set-up

 Hyalella, 7 to 14 days in age, are purchased from a reliable, experienced dealer and shipped to the laboratory via next day delivery service. Organisms are held in plastic bowls and allowed to acclimate to lab conditions for a minimum of six hours prior to test initiation.

Approved By:		
Unix Start 4/3/03	Ilem Carlan	6/4/03
Laboratory Manager (Date)	QA Supervisor	(Date)

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- 2. During the holding period animals are acclimated to lab conditions by replacing culture water with laboratory water using a slow drip into a holding bowl During acclimation, temperature changes should not exceed 2°C per 24-hr period.
- 3. Samples are checked in upon receipt. See procedural SOP for details on sediment sample check-in procedures. Sediment samples are sieved through a 0.5-mm screen to remove any resident organisms and debris. The sieved sediment is placed in a clean, labeled, plastic bag, and stored at 4°C until tests are initiated.
- 4. Interstitial porewater is collected from each sample for the measurement of ammonia, pH, and conductivity. Interstitial water is collected by centrifuging a subsample of whole sediment at 3000 rpm for 15 min. Overlying water present in sediment collection containers or bags may be collected for porewater analysis if the samples were originally placed in the containers with no overlying water. During shipment, sediments quickly settle, often leaving a layer of interstitial water on the sediment surface.

### 24-Hrs Prior to Test Initiation

- 5. Set up a randomization key using Excel software program.
- 6. Label tests chambers according to the randomization sheet and add 175 ml (approx. 2.5 centimeters) of the appropriate sediment to the labeled jars.
- 7. Prepare an additional test chamber for each test site. This surrogate will be used for water quality measurements during the test period.
- 8. Fill jars with 900-ml laboratory test water.
- 9. Place jars in the 23°C environmental chamber and let sit overnight to stabilize. Adjust light cycle in chamber to 16L/8D hour light cycle.

### B. Test Initiation - Day 0

- Measure and record water quality readings (D.O., conductivity, temperature, and pH) from the surrogate jars. Remove a subsample for ammonia analysis from each site and preserve with acid.
- 2. Place 10 animals into a 30-ml cup containing laboratory water.
- 3. A second count is performed to verify that there are 10 animals in each cup.
- 4. Randomly distribute two cups to each chamber for a total of 20 test animals per chamber by gently rinsing the contents of each cup into a test chamber with a laboratory water squirt bottle filled with moderately hard synthetic water.

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- 5. Aerate test chambers gently with 1-ml glass pipets placed in the water column with special care taken to prevent sediment disturbance.
- 6. Inspect all test chambers within two hours of initiation to ensure that no amphipods are trapped in the surface tension of the water. Replace any floating animals with new animals.
- 7. Obtain a mean initial dry weight of amphipods by placing 10 randomly selected animals on tared, dried weigh pans (5 replicates). Place pans in drying oven for 24 hours at 60°C. Weigh amphipods to the nearest 0.1mg.

## C. Daily Monitoring and Feeding Regime - Days 1 to 28

- Measure and record pH, D.O., conductivity, and temperature in surrogate chambers daily.
- 2. Collect a subsample of overlying water for ammonia analysis on days 0, 7, 14, 21, and 28 or as specified.
- Consult with sample specific requirements and perform overlying water change if conductivity or ammonia levels approach an unacceptable level (>30 mg/L).
- 4. Observe all chambers checking for potential problems such as uneven aeration and make appropriate adjustments.
- 5. Feed test chambers every 48-hours (e.g. Day 0, Day 2, Day 4, and Day 6, etc.) with 1 ml of Tetramin® solution per test chamber. Food consists of 2g TetraMin® blended into 100 ml lab water. If there is an accumulation of excess food on the sediment surface, suspend feeding to avoid fungal growth and degraded water quality in the test chambers.

### D. Test Termination - Day 28

- 1. Measure and record water quality (D.O., pH, conductivity, and temperature) in surrogate test chambers. Remove a subsample of overlying water for ammonia analysis from each site and preserve with sulfuric acid.
- 2. Gently suspend sediment in the test chambers and pour through a 0.5-mm cylindrical screen. Rinse away remaining sediment using laboratory water.
- Count and record the number of surviving animals.
- 4. Thoroughly rinse the surviving animals with deionized water, place them onto a tared, dried weighing pan with forceps, and dry at 60°C for 24 hours. Report total dry weight to the nearest 0.1 mg.

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### E. Reference Toxicant Testing

- 1. Conduct a concurrent 96-hour reference toxicant test with sediment tests to evaluate the health of the test organisms. Copper chloride is used as the reference toxicant. The testing concentrations for the reference toxicant are Control, 100, 200, 400, 800, and 1600  $\mu$ g/L.
- The LC<sub>50</sub> for survival should be within two standard deviations of the control chart mean for the laboratory.

#### IV. TEST ACCEPTABILITY CRITERIA

Mean survival of amphipods in the control at test termination must be 80 percent or greater.

### V. HEALTH AND SAFETY

Health and safety precautions and applicable regulations should be considered at all times. Gloves must always be worn when handling test sediments.

### VI. PERSONNEL

Only qualified technicians who have been properly trained and can demonstrate competency with these techniques are permitted to conduct this test.

### VII. QUALITY ASSURANCE REQUIREMENTS

Quality assurance practices encompass all aspects of testing including the collection, handling, and preparation of test organisms, samples, and dilution waters. Proper record keeping is required and concentrated efforts are made for complete documentation on a real-time basis.

#### VIII. REFERENCES

"Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates." ASTM E 1706-00, 2002.

"ASTM Standards on Aquatic Toxicology and Hazard Evaluation." ASTM publication Code Number: 03-547093-16, 1993.

"Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates, Second Edition." USEPA, EPA 600/R-99/064, 2000

"Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S.-Testing Manual." Environmental Protection Agency/Corps of Engineers. EPA-823-B-98-004, 1998.

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"Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms." USEPA, EPA/600/4-90/027F, 1993.